

Early origins of inflammation: microbial exposures in infancy predict lower levels of C-reactive protein in adulthood

Thomas W. McDade^{1,2,*}, Julienne Rutherford³, Linda Adair⁴
and Christopher W. Kuzawa^{1,2}

¹Department of Anthropology, and ²Cells to Society (C2S): The Center on Social Disparities and Health, Institute for Policy Research, Northwestern University, Evanston, IL 60208, USA

³Department of Oral Biology, College of Dentistry, University of Illinois at Chicago, Chicago, IL, USA

⁴Department of Nutrition, Carolina Population Center, University of North Carolina, Chapel Hill, NC, USA

Ecological factors are important determinants of the development and function of anti-pathogen defences. Inflammation is a central part of innate immunity, but the developmental factors that shape the regulation of inflammation are not known. We test the hypothesis that microbial exposures in infancy are associated with high sensitivity C-reactive protein (CRP) in adulthood using prospective data from a birth cohort in the Philippines ($n = 1461$). Lower birth weight was associated with increased CRP, consistent with a role for inflammation in the widely documented inverse relationship between birth weight and adult cardiovascular diseases. In addition, higher levels of microbial exposure in infancy were associated with lower CRP. These associations were independent of socioeconomic status, measures of current body fat and other health behaviours. We conclude that measures of microbial exposure and nutrition during the pre-natal and early post-natal periods are important predictors of CRP concentration in young adulthood. We speculate that the development of anti-inflammatory regulatory networks in response to early microbial exposure represents plasticity in the development of anti-pathogen defences, and that this process may help explain the low CRP concentrations in this population.

Keywords: inflammation; infectious disease; cardiovascular disease; developmental origins of adult disease; ecological immunology

1. INTRODUCTION

Recent analyses of historical demographic data have led to the proposal that human life expectancy gains over the past 250 years in industrialized settings are due in part to reduced exposure to inflammation across the life course. According to this hypothesis, early infectious exposures result in a ‘cohort morbidity phenotype’ characterized by chronic activation of inflammatory pathways, thereby increasing risk for morbidity and mortality later in life (Finch & Crimmins 2004; Crimmins & Finch 2006). This hypothesis draws on a rapidly growing body of research demonstrating that high sensitivity C-reactive protein (CRP)—a key biomarker of inflammation—is positively associated with incident cardiovascular disease (CVD) (Ridker *et al.* 1998), type 2 diabetes (Pradhan *et al.* 2001), the metabolic syndrome (Ridker *et al.* 2003), late-life disability (Kuo *et al.* 2006) and mortality (Jenny *et al.* 2007) in older adults.

The idea that environmental exposures early in life condition risk for disease in adulthood first emerged from group-level analyses of vital statistics showing that community death rates in infancy and childhood predict life expectancy within birth cohorts, independently of current living conditions (Kermack *et al.* 1934; Forsdahl

1977). Pre-natal under nutrition—as indicated most commonly by lower birth weight—has since been the focus of efforts to explain these cohort effects (Barker & Osmond 1986), while more recent research including a wider range of human populations and experimental animal models has demonstrated that pre-natal and early post-natal nutritional stressors increase risk for cardiovascular and metabolic diseases later in life through developmental modifications to physiological systems, organ growth and metabolism (Barker 1994; Gluckman *et al.* 2008). In light of this literature, the inflammation hypothesis requires careful evaluation to identify whether early infectious exposures are associated with physiological function, morbidity and mortality later in life, and whether these associations are independent of other early life exposures. No studies of which we are aware have investigated post-natal infectious or microbial exposures in relation to inflammation in individuals that were subsequently followed longitudinally into adulthood.

An alternative, and not necessarily contradictory, adaptationist approach recognizes that anti-pathogen defences are energetically costly to the organism, and that systems of defence can be expected to develop in response to ecological factors across the life course (Sheldon & Verhulst 1996; Schulenburg *et al.* 2009). CRP is an important component of innate immunity,

* Author for correspondence (t-mcdade@northwestern.edu).

and is involved in activating complement, promoting phagocytic activity and opsonizing bacteria, fungi and parasites. It is produced by hepatocytes in response to pro-inflammatory cytokines, including IL-6, IL-1 and TNF- α (Ballou & Kushner 1992; Libby *et al.* 2002). As part of the acute phase response, concentrations of CRP increase dramatically in response to a wide range of pathogens, providing a rapid first line of defence while slower adaptive immune defences come online. For this reason, CRP has historically been used as a non-specific marker of infection, but the recent advent of high sensitivity assays has drawn attention to the important role that chronic, low-grade inflammation may play in the pathophysiology of a wide range of chronic diseases (Ross 1999). The vast majority of this work has been conducted in relatively affluent settings with low levels of infectious disease, and it has focused on excess body fat—visceral adipose tissue in particular—as a primary source of pro-inflammatory cytokines that increase production of CRP (Lyon *et al.* 2003; Rexrode *et al.* 2003).

However, developmental plasticity and ecological responsiveness are key features of the human immune system, and nutritional as well as microbial exposures early in life are prime drivers of immune development that have implications for function in adulthood (McDade 2003). Evidence in support of the ‘hygiene hypothesis’ provides an example: reduced exposure to microbes in infancy/childhood owing to rising standards of sanitation and hygiene has been associated with elevated rates of atopic diseases, as well as other pathologies related to immune dysregulation, later in life (Strachan 1989; Rook & Stanford 1998; Illi *et al.* 2001). Similarly, in prior research, we have shown that low birth weight was associated with reduced antibody responsiveness to vaccination in adolescence, whereas higher levels of diarrhoea morbidity in infancy predicted enhanced antibody responsiveness (McDade *et al.* 2001). From this perspective, infectious exposures represent important ecological inputs that guide the development of immune defences, and in the absence of such inputs, poorly regulated or self-directed activity may be more likely to emerge. As such, one might hypothesize that early microbial exposures are negatively associated with inflammation in adulthood, in contrast to the cohort morbidity hypothesis. Research on CRP in a population characterized by higher levels of exposure to infectious disease is necessary to address this question, and to complement current research on the determinants of inflammation conducted in affluent settings with low burdens of infection.

Here, we test the hypothesis that microbial and infectious exposures in infancy are associated with CRP in young adulthood using data from a large prospective birth cohort study in the Philippines. The study began in 1983 when the participants were *in utero*, and it included detailed follow-up surveys throughout infancy to assess infectious morbidity, infant feeding, growth and other characteristics, as well as several subsequent follow-up surveys across childhood, adolescence and into adulthood. The Philippines is a lower middle income nation in the midst of significant economic, dietary and lifestyle transitions, and it exemplifies current trends towards increasing rates of overweight, CVDs and the metabolic syndrome globally (Adair 2004;

Tanchoco *et al.* 2003). However, infectious diseases still account for significant burdens of morbidity and mortality, and episodes of diarrhoea were frequent when the cohort was in infancy (VanDerlice *et al.* 1994; Rice *et al.* 2000).

Prior cross-sectional analyses of CRP in adulthood in this cohort have revealed low concentrations compared with western populations, as well as different patterns of association between body fat and CRP, raising the possibility that distinct ecological exposures early in life may have lasting impact (McDade *et al.* 2008, 2009). We evaluate this possibility by taking advantage of the detailed longitudinal data to consider whether measures of early nutrition and infectious exposures predict elevated CRP in adulthood, adjusting for a range of potentially confounding and moderating influences.

2. MATERIAL AND METHODS

(a) *Participants and data collection*

The Cebu Longitudinal Health and Nutrition Survey (CLHNS) began in 1983 with the recruitment of 3327 pregnant women representative of the childbearing population in Cebu City (Cebu Study Team 1991). A single stage cluster sampling procedure was used to randomly select 17 urban and 16 rural neighbourhoods in Cebu, and households were surveyed to locate all pregnant women. The women and their children have been followed through multiple rounds of data collection since 1983, including the most recent survey conducted in 2005. The 2005 survey included 1885 index children, 1486 of whom provided complete anthropometric, CRP and interview data for these analyses. An additional 25 women pregnant at the time of the survey were not included in the analyses owing to the effect of pregnancy on inflammation, yielding a final sample size of 1461.

Participants provided information on household demographics, economic activities and resources, environmental quality and health behaviours in face-to-face interviews conducted in their homes. Interviewers also provided assessments of household and neighbourhood attributes. Standard procedures (Lohman *et al.* 1988) were implemented in the home to collect anthropometric measures of standing height (without footwear), weight (in light clothing), skinfold thicknesses and waist circumference. All data were collected under conditions of informed consent with institutional review board approval from the University of North Carolina, Chapel Hill.

We evaluated how our sample differed from the original cohort as assessed when the study started in 1983. Compared with those lost to follow-up, participants remaining in the study had higher mean birth weights (mean (s.e.) difference = 52.7 (15.9) g, $p < 0.001$), were born to mothers with less formal education (0.37 (0.13) years, $p < 0.01$) and had higher frequencies of infection in the first year of life (respiratory infection: 0.61 (0.06) episodes, $p < 0.001$; diarrhoea: 0.20 (0.04) episodes, $p < 0.001$). They were also more likely to come from families that owned their homes in 1983 (74.1 versus 57.3%, $p < 0.001$), and were more likely to live in rural communities (25.6 versus 21.5%, $p < 0.01$). Participants did not differ with respect to household income or assets at baseline.

Attrition in the CLHNS is due primarily to out migration. Of the pregnant women who were initially asked to participate in 1983 during the complete census of selected

neighbourhoods, fewer than 4 per cent refused. The study enrolled 3327 women during pregnancy, 3080 of whom gave birth to a single live infant, comprising the core sample for subsequent follow-up. Individuals not followed up had multiple births ($n = 26$), stillbirth or miscarriage ($n = 42$), refused participation after baseline enrollment ($n = 17$) or migrated outside of the Cebu area ($n = 136$). Rates of refusal for subsequent surveys were low (5–10%), with loss-to-follow-up due primarily to individuals relocating outside of the Metro Cebu area. As a result, participants in the 2005 survey are selective of households that are less mobile compared with all participants in the original cohort.

(b) CRP analysis

Blood samples were collected into EDTA-coated vacutainer tubes in the participants' homes in the morning after an overnight fast. Blood samples were kept in coolers on ice packs for no more than 2 h and were then centrifuged to separate plasma prior to freezing at -70°C . Samples were express-shipped to Northwestern University on dry ice and stored frozen at -80°C until analysis. CRP concentrations were determined using a high sensitivity immunoturbidimetric method (Synchron LX20, lower detection limit: 0.1 mg l^{-1}).

(c) Independent variables

Information on infectious morbidity was collected during the first two years as part of in-home interviews conducted at bimonthly intervals following birth. Mothers were asked whether their infant had shown symptoms of diarrhoea or respiratory infection during the week preceding the interview. We constructed separate variables summing the number of bimonthly intervals in which individuals had diarrhoea or respiratory infection. In order to consider the potential impact of timing of exposure, separate morbidity variables were constructed for 2–12 and 14–24 months, inclusive.

Following prior research in the Philippines and elsewhere (VanDerslice *et al.* 1994; Nurgalieva *et al.* 2002; Prado *et al.* 2003), we considered several household-based measures to estimate the level of exposure to infectious microbes in infancy. These measures included the following, assessed during baseline interviews in 1983: household density (number of persons/number of rooms), level of faecal contamination of the yard outside the home (based on interviewer observation; 0 = no faeces, 4 = heavy contamination), type of toilet (no toilet or pit versus flush/water sealed) and source of drinking water (closed sources: bottled, piped municipal supply, closed well with pump, versus open sources: uncovered well, spring, river, rain). Exposure to animal faeces in the home was assessed by summing the number of bimonthly intervals that the interviewer observed that the infant was crawling, and that animals were present in the home.

Separate indicator variables were defined to indicate birth in the dry season or the rainy season. The dry season was defined as February to April. The rainy season was defined as June to October. Prior analyses have shown that rainfall, poor drainage and water supplies contaminated by heavy rains are associated with spread of pathogens and infectious morbidity in Cebu (Moe *et al.* 1991; VanDerslice *et al.* 1994). The frequency of diarrhoea (1.4 versus 1.0 episodes, $p < 0.001$) and respiratory infection (4.4 versus 4.2, $p < 0.05$) in the 12 months after delivery was significantly higher for individuals born in the dry season, suggesting

higher levels of microbial exposure in early infancy for these individuals.

Measures of the pre-natal environment included birth weight, gestational age and parity. Additional post-natal measures included growth in length and weight during the first two years, and the duration of breastfeeding (exclusive and total). Anthropometric measures and breastfeeding information were collected at each bimonthly interview during the first two years following birth.

In order to assess infectious morbidity at the time of blood collection, we asked participants if they were currently experiencing any symptoms of infection. Symptoms included runny nose, cough, fever, diarrhoea, sore throat as well as the more general categories of 'flu,' 'cold,' and 'sinusitis'. Responses were used to construct a single variable indicating the presence of any infectious symptoms at the time of blood collection.

Other adult characteristics included anthropometric measures of adiposity (waist circumference, sum of triceps, subscapular and supra-iliac skinfold thicknesses), individual health behaviours shown previously to be related to CRP (smoking, alcohol consumption, oral contraceptive use) and multiple measures of socioeconomic status: household income, household assets and educational attainment. Following prior cross-sectional analyses of the predictors of CRP in this sample (McDade *et al.* 2009), we also constructed a household pathogen exposure variable based on five measures, each scored on a three-point scale (0 = low exposure, 1 = moderate, 2 = high) and assessed at the time of CRP measurement: cleanliness of the food preparation area, means of garbage disposal, presence of excrement near the house, level of garbage and excrement present in the neighbourhood surrounding the household.

(d) Data analysis

We tested the hypothesis that infectious exposures in infancy are associated with inflammation in adulthood in two ways. First, we used a series of logistic regression models predicting the likelihood of having CRP concentration in the top tertile of the sample distribution ($\geq 0.7\text{ mg l}^{-1}$). The tertile approach has been used previously in several population-based studies of CRP (Danesh *et al.* 2000), and our application of this approach facilitates comparison with prior research in other settings. A cut-point approach also allows us to consider whether associations between early environments and adult CRP concentration are threshold in nature, rather than continuous.

Second, we evaluated a series of regression models investigating log-transformed CRP as a continuous dependent variable. We applied Tobit regression models for censored data to account for non-normality in the distribution of log-CRP values. Left censoring of the distribution is due to the large number of observations with values below the lower detection limit of the CRP assay. The application of ordinary least-squares regression procedures would probably result in biased and unstable parameter estimates, whereas Tobit regression takes into account the censored nature of the distribution to provide more reliable parameter estimates (Greene 2000). Observations below the detection limit are included in all Tobit models (as they are for the logistic regression models).

For both logistic and censored regression models, individuals with $\text{CRP} > 10\text{ mg l}^{-1}$ were removed from the analyses based on recommendations issued by a recent joint scientific

Table 1. Descriptive statistics for female and male participants. Mean (s.d.) values are presented for continuous variables ($n = 1461$).

	female ($n = 672$)	male ($n = 862$)	total ($n = 1534$)
assessed in infancy			
birth weight (kg)	2.991 (0.402)	3.029 (0.433)	3.012 (0.420)
gestation length (weeks)	39.2 (2.5)	38.8 (2.4)	39.0 (2.5)
weight gain, 1st year (kg)	4.678 (0.860)	5.217 (0.934)	4.978 (0.945)
mother's education (years)	7.3 (3.6)	7.5 (3.7)	7.4 (3.6)
household income (pesos)	278.1 (411.7)	286.3 (539.0)	282.7 (486.8)
duration exclusive breastfeeding (days)	60.2 (38.9)	57.0 (39.5)	58.4 (39.3)
assessed in adulthood			
age (years)	20.9 (0.3)	20.9 (0.3)	20.9 (0.3)
education (years)	11.6 (3.3)	10.5 (3.8)	10.9 (3.6)
waist circumference (cm)	68.1 (7.7)	72.1 (7.5)	70.3 (7.8)
sum of three skinfold measures (mm)	62.3 (19.6)	37.6 (18.1)	48.4 (22.4)
symptoms of infection (%)	14.6	13.7	14.1
oral contraceptive use (%)	3.7		
CRP (median, 25th, 75th percentile)	0.2 (0.1, 0.9)	0.3 (0.1, 0.9)	0.2 (0.1, 0.9)

statement from the American Heart Association and the Centers for Disease Control and Prevention (Pearson *et al.* 2003). Concentrations of CRP above 10 mg l^{-1} are presumed to be the result of acute inflammatory processes, although recent research has suggested that CRP concentrations above 10 mg l^{-1} are also predictive of CVD risk (Mueller *et al.* 2002). We therefore conducted a final series of analyses using the entire sample. All statistical analyses were conducted with STATA for Windows, v. 10 (StataCorp, College Station, TX).

Analyses proceeded in three stages. First, we considered bivariate associations between adult CRP and multiple measures of microbial exposure and infectious disease symptoms in the first two years of infancy (unadjusted models). We also considered birth weight, post-natal growth and breastfeeding duration as additional predictors of adult CRP. Second, we evaluated these variables simultaneously in regression models including gender to evaluate whether significant bivariate associations were independent of other, likely correlated, aspects of the early environment (model 1). Lastly, we considered whether these associations were modified when adjusting for adult characteristics known to affect CRP concentration (model 2); $\alpha < 0.05$ was used as the criterion for statistical significance, and variables with $p < 0.10$ were retained to explore trends in the data.

3. RESULTS

The median CRP concentration for the entire sample was 0.2 mg l^{-1} , and was not significantly different by gender (table 1). Fifty-two participants (3.3%) had concentrations of CRP $> 10 \text{ mg l}^{-1}$. The presence of infectious disease symptoms at the time of blood collection was the only significant predictor of CRP $> 10 \text{ mg l}^{-1}$ (OR = 3.64; 95% CI = 2.00, 6.61; $p < 0.001$). Unless noted otherwise, these individuals were excluded from subsequent analyses. No anthropometric, socioeconomic or other environmental quality variables were significantly related to this level of CRP.

Bivariate analyses did not reveal any significant positive associations between higher levels of microbial exposures in infancy and elevated CRP in young adulthood (table 2). Three variables were significantly associated

Table 2. Bivariate associations between elevated CRP in young adulthood and measures of infectious exposures in infancy (excluding individuals with CRP $> 10 \text{ mg l}^{-1}$). Mean (s.d.) values are presented for continuous variables; percentages are presented for categorical variables. Two-sample t -tests (continuous variables) and Pearson χ^2 -tests (categorical variables) were used to evaluate differences between groups with CRP < 0.7 and CRP $\geq 7 \text{ mg l}^{-1}$.

	CRP $< 0.7 \text{ mg l}^{-1}$	CRP $\geq 0.7 \text{ mg l}^{-1}$
diarrhoea, 1st year (no. episodes)	1.12 (1.10)	1.05 (0.99)
diarrhoea, 2nd year	1.20 (1.20)	1.06 (1.14)*
respiratory infection, 1st year (no. episodes)	4.37 (1.35)	4.31 (1.33)
respiratory infection, 2nd year	4.33 (1.42)	4.37 (1.40)
level of faecal contamination near house (0–4)	0.87 (0.99)	0.85 (1.01)
animal faeces in house, 6–12 months (no. bimonthly intervals)	1.33 (1.30)	1.08 (1.20)**
open water source (%)	89.0	87.9
flush toilet (%)	45.1	44.8
household density (persons/room)	2.55 (1.55)	2.50 (1.48)
dry season birth (%)	22.3	16.8*

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

with CRP contrary to the inflammation hypothesis: individuals with elevated CRP as adults were exposed to lower levels of animal faeces in the home, had fewer episodes of diarrhoea in their second year and were less likely to be born in the dry season. In addition, birth weight was a

Table 3. Results of maximum-likelihood logistic regression models predicting the probability of CRP in the top tertile ($\geq 0.7 \text{ mg l}^{-1}$), excluding individuals with CRP $> 10 \text{ mg l}^{-1}$ ($n = 1409$).^a

	unadjusted		model 1		model 2	
	OR	95% CI	OR	95% CI	OR	95% CI
diarrhoea, 1st year (no. episodes)	0.94	0.84, 1.05	0.98	0.87, 1.10	0.98	0.87, 1.11
diarrhoea, 2nd year	0.90*	0.81, 0.99	0.90†	0.81, 1.00	0.89*	0.80, 0.99
resp. infect., 1st year	0.97	0.89, 1.06	0.97	0.88, 1.06	1.00	0.90, 1.10
resp. infect., 2nd year	1.02	0.94, 1.11	1.05	0.96, 1.15	1.00	0.91, 1.10
faeces near house (0–4)	1.00	0.82, 1.21	1.04	0.85, 1.28	1.02	0.82, 1.26
animal faeces in house (no. intervals)	0.86**	0.78, 0.94	0.86**	0.78, 0.94	0.87**	0.79, 0.96
open water source (0, 1)	0.90	0.63, 1.28	0.87	0.59, 1.26	0.91	0.60, 1.38
flush toilet (0, 1)	0.99	0.79, 1.25	0.95	0.74, 1.21	0.94	0.72, 1.24
household density (persons/room)	0.98	0.91, 1.06	0.98	0.90, 1.06	0.96	0.89, 1.05
dry season birth (0, 1)	0.70*	0.52, 0.94	0.70*	0.51, 0.94	0.67*	0.49, 0.92
birth weight (kg)	0.78†	0.59, 1.03	0.77†	0.59, 1.02	0.71*	0.53, 0.95

^aModel 1 includes variables listed in table 3 and adjusts for gender. Model 2 includes these variables, but also adjusts for maternal education, household income at birth, current household income, current waist circumference, skinfold thickness, oral contraceptive use, symptoms of infection at the time of blood collection and household pathogen exposure.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

† $p < 0.10$.

significant predictor of adult CRP, with lower birth weights associated with higher CRP. Measures of post-natal growth, breastfeeding, gestational age and parity were not associated with adult CRP, nor did they modify associations with other variables. These variables were therefore not included in subsequent analyses.

We next considered microbial exposure variables, as well as birth weight, in multiple regression models to evaluate their relative independence in predicting CRP, and to evaluate whether bivariate associations were altered after adjusting for adult characteristics known to influence CRP concentration. In logistic regression models predicting the odds of elevated CRP, associations between microbial exposures in infancy and adult CRP were essentially unchanged (table 3, models 1 and 2). Each additional reported episode of diarrhoea in the second year of life was associated with an 11 per cent reduction in the odds of elevated CRP in young adulthood. Similarly, for each bimonthly interval between 6 and 12 months of age where exposure to animal faeces was likely, the odds of elevated CRP in adulthood were 13 per cent lower. Lastly, individuals born in the dry season were 33 per cent less likely to have elevated CRP as a young adult. Birth weight also remained a significant predictor of CRP in adulthood, with each kilogram increase in birth weight associated with a 29 per cent reduction in the odds of elevated CRP. These associations were independent of body fat, health behaviours and infectious disease measures evaluated at the time of blood collection in young adulthood.

Analysis of CRP as a continuous outcome yielded similar, though less robust, results (table 4). Higher levels of exposure to animal faeces in infancy were associated with significantly lower concentrations of CRP in adulthood. More frequent episodes of diarrhoea in infancy and birth in the dry season were both associated with lower CRP, although these associations were not statistically significant in bivariate or fully adjusted models. Larger birth weight was significantly associated with lower CRP concentration in adulthood.

Figures 1 and 2 present the predicted probability of elevated CRP and the predicted concentration of CRP, respectively, in relation to microbial exposures early in life to demonstrate the relative strength of these associations. Moving from the highest to the lowest levels of diarrhoea morbidity and exposure to animal faeces both increased the predicted probability of elevated CRP by a factor of 1.4. Dry season birth was associated with a comparable level of reduction in the probability of elevated CRP. A 1 kg decrease in birth weight was associated with a 1.3-fold increase in the probability of elevated CRP in young adulthood. In terms of CRP concentration, a 1 kg decrease in birth weight was associated with 21 per cent higher CRP, while low levels of faecal exposure were associated with 24 per cent higher CRP. Birth outside of the dry season and low levels of diarrhoea were each associated with 11 per cent higher concentrations of CRP, although as noted before these differences were not statistically significant.

We re-ran our models to include individuals with CRP greater than 10 mg l^{-1} . Logistic regression results were essentially the same. Tobit regression results were also similar, with the exception of a strengthening of the association between adult CRP concentration and birth in the dry season ($B = -0.11$, $\text{s.e.} = 0.066$, $p = 0.10$). Results for both sets of models were unchanged when individuals reporting current symptoms of infection ($n = 198$) as well as individuals with CRP $> 10 \text{ mg l}^{-1}$ were removed from the model.

4. DISCUSSION

We find no evidence for positive associations between measures of microbial exposures in infancy and inflammation in adulthood. Rather, we find several negative associations between three distinct measures of microbial exposure early in life and CRP production in young adulthood, independent of a wide range of potentially confounding variables. We investigated this issue in an ongoing, longitudinal study that began collecting data

Table 4. Results of Tobit regression models predicting log-transformed CRP, excluding individuals with CRP > 10 mg l⁻¹ (*n* = 1409).^a

	unadjusted		model 1		model 2	
	<i>B</i>	s.e.	<i>B</i>	s.e.	<i>B</i>	s.e.
diarrhoea, 1st year (no. episodes)	-0.028	0.024	-0.021	0.025	-0.018	0.024
diarrhoea, 2nd year	-0.029	0.021	-0.029	0.023	-0.025	0.021
resp. infect., 1st year	-0.005	0.019	-0.009	0.020	0	0.019
resp. infect., 2nd year	0.017	0.018	0.024	0.019	0.006	0.018
faeces near house (0-4)	0.006	0.043	0.019	0.044	0.011	0.042
animal faeces in house (no. intervals)	-0.075***	0.020	-0.073***	0.020	-0.063**	0.019
open water source (0, 1)	-0.074	0.079	-0.099	0.081	-0.061	0.081
flush toilet (0, 1)	0.016	0.051	-0.004	0.053	-0.007	0.053
household density (persons/room)	0.011	0.016	0.011	0.017	0.004	0.016
dry season birth (0, 1)	-0.083	0.063	-0.078	0.063	-0.082	0.060
birth weight (kg)	-0.137*	0.060	-0.142*	0.060	-0.171**	0.057
constant			-0.158	0.232	-0.822†	0.469

^aModel 1 includes variables listed in table 4 and adjusts for gender. Model 2 includes these variables, but also adjusts for maternal education, household income at birth, current household income, current waist circumference, skinfold thickness, oral contraceptive use, symptoms of infection at the time of blood collection and household pathogen exposure.

**p* < 0.05.

***p* < 0.01.

****p* < 0.001.

†*p* < 0.10.

while participants were *in utero*, and that includes detailed information on the same set of individuals over a 22 year period. This is a major strength of our analysis, and increases our confidence that the reported associations are not due to confounding or omitted variable bias.

These results run counter to the 'cohort morbidity phenotype' hypothesis (Finch & Crimmins 2004; Crimmins & Finch 2006), which predicts positive associations between infections at younger ages and inflammation and CVD risk at older ages. However, our results do not constitute a direct refutation, and our study should not be interpreted as a direct test of the hypothesis. The nature of pathogenic exposures in the Philippines in the 1980s and in Western Europe in the eighteenth and nineteenth centuries may be qualitatively distinct, making it difficult to compare results from 22 years of prospective data with results from two centuries of historical demographic data. Furthermore, it is possible that the hypothesized positive associations between infectious exposures and inflammation will emerge after young adulthood, when indicators of cardiovascular risk tend to accumulate. Lastly, positive associations between infections in childhood and adult morbidity have been reported in other populations, suggesting that the timing, type and intensity of infectious exposures may be critical determinants of their long-term effects on inflammation (Blackwell *et al.* 2001; Kemp & Björkstén 2003). Unfortunately, our study lacks intensive morbidity data beyond age two years, preventing us from considering whether patterns of association between infectious disease and adult CRP are different in infancy and childhood.

Negative associations between microbial exposures in infancy and inflammation in adulthood are broadly consistent with the hygiene or 'old friends' hypothesis, in which low levels of pathogen exposure early in life bias immune development and regulatory processes in ways that increase the likelihood of inflammatory conditions

such as allergy, asthma and autoimmune disease later in life (Rook & Stanford 1998; Yazdanbakhsh *et al.* 2002; Rook *et al.* 2004; Radon *et al.* 2007). Increased rates of these and other diseases linked to inflammation are commonly observed following economic development in lower income, pre-epidemiologic transition populations. Our results are consistent with these observations, and point to a potentially important mechanism.

Furthermore, we find stronger associations between adult CRP and proxy measures of microbial exposure than we do with measures of infectious morbidity in infancy. This pattern of results supports a central component of the old friends hypothesis: chronic exposure to harmless microbes common throughout human evolutionary history is critical to immune development, and the absence of such exposure is the key factor leading to immune dysregulation (Rook *et al.* 2004, 2009). Full blown, symptomatically experienced infectious disease is not necessary for this protective effect, only engagement with a rich microbial environment. Common bacteria may play particularly important roles in this process, and this may explain in part why we find a strong association between adult CRP and exposure to animal faeces in infancy, and no association between CRP and measures of likely viral exposure like household density. Unfortunately, our surrogate measures of pathogen exposure do not allow detailed characterization of the microbial environment, particularly with respect to identifying specific classes of pathogens.

Recent research has underscored the importance of plasticity and ecological responsiveness in the development and function of the immune system, and microbial exposures in infancy may comprise a critical set of inputs that promote the development of regulatory T cells and effective anti-inflammatory regulatory networks (Yazdanbakhsh *et al.* 2002; Rook *et al.* 2004; McDade 2005). We speculate that a lack of such inputs may lead to a pro-inflammatory immunophenotype that

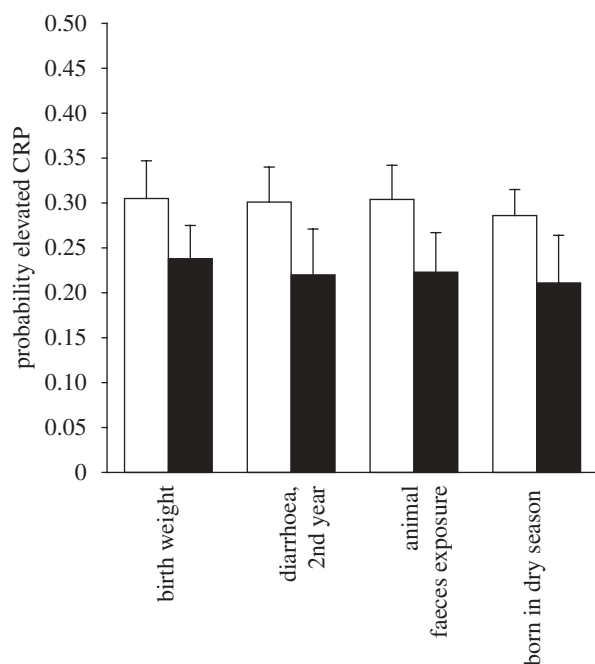


Figure 1. Probability of elevated CRP in relation to birth weight and pathogen exposure in infancy. Note: values are predicted probability of CRP in top tertile for the entire sample (excluding individuals with $\text{CRP} \geq 10 \text{ mg l}^{-1}$) based on coefficients from table 3, model 2, with upper bound of 95% CI. Low and high values for predictors were set as follows: birth weight (2.5 and 3.5 kg); diarrhoea, 2nd year (0, 3 or more episodes); animal faeces exposure (0, 3 or more intervals); born in dry season (no, yes). Original values were retained for other covariates. Unshaded box, low/no; shaded box, high/yes.

is more likely to produce CRP in response to inflammatory stimuli, or less likely to downregulate CRP production following activation. Epigenetic modifications to regulatory genes related to CRP are a potential mechanism linking early exposures with adult phenotypes that are worthy of further investigation (Bateson *et al.* 2004; Waterland & Michels 2007).

These mechanisms may explain, in part, the exceptionally low concentrations of CRP in our sample from the Philippines. In the USA, median CRP has been reported to be 2 mg l^{-1} for adults 17 years and older (King *et al.* 2003), and 0.9 mg l^{-1} for 20–29-year-old men (Ford *et al.* 2003). In Scotland and Germany, geometric mean concentrations for 25–34-year-olds ranged from 0.81 to 1.25 mg l^{-1} for men and women (Hutchinson *et al.* 2000). Much of the four to tenfold difference in CRP concentrations between these nations and the Philippines may be attributed to differences in body fat, with higher levels of overweight and obesity in the USA and Europe. However, in prior analyses, we have found that even in the ranges of the body fat distribution that overlap between Cebu and the USA, age- and gender-matched levels of CRP were elevated in the USA (McDade *et al.* 2009). To the extent that levels of microbial exposure in infancy were higher in the Philippines than in the USA and Europe 20 years ago, lower concentrations of CRP among Filipino adults are consistent with the hypothesis that early microbial exposures have lasting anti-inflammatory effects. And to the extent that a pro-inflammatory phenotype contributes to the development

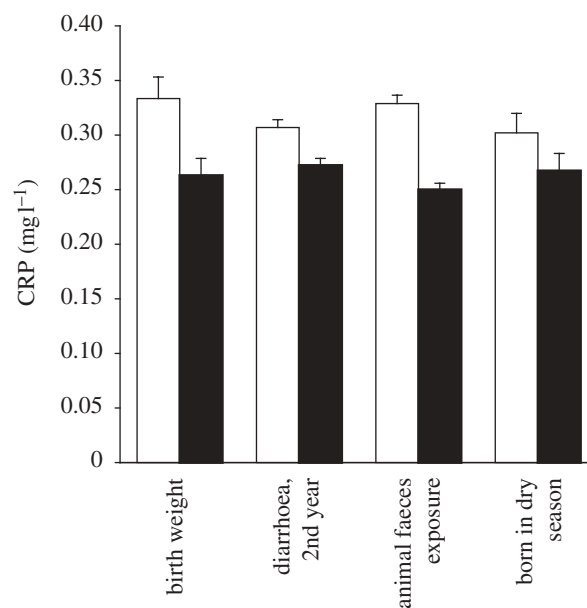


Figure 2. Concentration of CRP in relation to birth weight and pathogen exposures in infancy. Note: values are predicted concentration of CRP in the entire sample (excluding individuals with $\text{CRP} \geq 10 \text{ mg l}^{-1}$) based on coefficients from table 4, model 2. Predicted values are presented with the standard error of the estimate. Low and high values for predictors were set as follows: birth weight (2.5 and 3.5 kg); diarrhoea, 2nd year (0, 3 or more episodes); animal faeces exposure (0, 3 or more intervals); born in dry season (no, yes); adult waist circumference ($\pm 1 \text{ s.d.}$); adult skinfold thickness ($\pm 1 \text{ s.d.}$). Original values were retained for other covariates. Unshaded box, low/no; shaded box, high/yes.

of CVD, findings from the Philippines raise the intriguing possibility that early microbial exposures may be protective.

To the best of our knowledge, this study is the first to document an association between birth weight and CRP in adulthood using data from a prospective birth cohort. This finding is consistent with a recent study in Scotland, in which CRP concentrations in adulthood (age 30–59 years) were negatively associated with birth weight as recalled by study participants (Sattar *et al.* 2004). In addition, low birth weight has recently been associated with elevated CRP in five-year-old children in Bangladesh, suggesting that effects of birth weight on CRP may emerge in childhood (Raqib *et al.* 2007). The negative association between birth weight and adult CRP is also consistent with the large number of studies linking lower birth weights with the adult onset of CVD and diabetes as well as indicators of risk including blood pressure, total and HDL cholesterol, and insulin resistance (Rich-Edwards *et al.* 1999). Inflammation may therefore be an important mechanism that contributes to the consistently documented association between lower birth weight and increased risk for cardiovascular and metabolic disease later in life (Barker & Osmond 1986; Barker 1994; Gluckman *et al.* 2008).

Limitations of our study include the use of a single CRP measure, which makes it more difficult to differentiate acute episodes of inflammation from chronic, low-grade increases in CRP concentration. We also rely on proxy—rather than direct—measures of microbial exposure and bimonthly morbidity interviews to capture

the quality of the early environment with respect to infectious exposures. While neither set of variables provides a complete picture of infectious or microbial burden, the study is unique in its wide range of measures and the prospective nature of data collection. However, as with any cohort, attrition in our study has resulted in a sample that is no longer representative of the original cohort. Out migration accounts for the majority of lost participants, and caution is therefore warranted when applying these findings to other populations. Lastly, the significance of elevated CRP for cardiovascular and metabolic disease risk in this population is not clear, particularly given the relatively low concentrations compared with western populations. Future follow-up surveys will be required to determine whether reduced concentrations of CRP, and the antecedent conditions associated with the regulation of inflammation, shape the course of chronic degenerative disease in the Philippines as well as other populations in the midst of the nutrition transition.

Funding for this study was provided by the National Institutes of Health (RO1 HL085144; 5 RO1 TW05596); biomarker data collection was supported by pilot funds from the Interdisciplinary Obesity Center (RR20649) and the Center for Environmental Health and Susceptibility (ES10126; project 7-2004-E).

REFERENCES

- Adair, L. S. 2004 Dramatic rise in overweight and obesity in adult Filipino women and risk of hypertension. *Obes. Res.* **12**, 1335–1341. (doi:10.1038/oby.2004.168)
- Ballou, S. P. & Kushner, I. 1992 C-reactive protein and the acute phase response. *Adv. Intern. Med.* **37**, 313–336.
- Barker, D. J. 1994 *Mothers, babies and diseases in later life*. London, UK: BMJ Publishing Group.
- Barker, D. J. P. & Osmond, C. 1986 Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* **327**, 1077–1081. (doi:10.1016/S0140-6736(86)91340-1)
- Bateson, P. *et al.* 2004 Developmental plasticity and human health. *Nature* **430**, 419–421. (doi:10.1038/nature02725)
- Blackwell, D. L., Hayward, M. D. & Crimmins, E. M. 2001 Does childhood health affect chronic morbidity in later life? *Soc. Sci. Med.* **52**, 1269–1284. (doi:10.1016/S0277-9536(00)00230-6)
- Cebu Study Team 1991 Underlying and proximate determinants of child health: The Cebu Longitudinal Health and Nutrition Study. *Am. J. Epidemiol.* **133**, 185–201.
- Crimmins, E. M. & Finch, C. E. 2006 Infection, inflammation, height, and longevity. *Proc. Natl Acad. Sci. USA* **103**, 498–503. (doi:10.1073/pnas.0501470103)
- Danesh, J., Whincup, P., Walker, M., Lennon, L., Thomson, A., Appleby, P., Gallimore, J. R. & Pepys, M. B. 2000 Low grade inflammation and coronary heart disease: Prospective study and updated meta-analyses. *Br. Med. J.* **321**, 199–204. (doi:10.1136/bmj.321.7255.199)
- Finch, C. E. & Crimmins, E. M. 2004 Inflammatory exposure and historical changes in human life-spans. *Science* **305**, 1736–1739. (doi:10.1126/science.1092556)
- Ford, E. S., Giles, W. H., Myers, G. L., Rifai, N., Ridker, P. M. & Mannino, D. M. 2003 C-reactive protein concentration distribution among US children and young adults: findings from the National Health and Nutrition Examination Survey, 1999–2000. *Clin. Chem.* **49**, 1353–1357. (doi:10.1373/49.8.1353)
- Forsdahl, A. 1977 Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? *Br. J. Prev. Soc. Med.* **31**, 91–95.
- Gluckman, P. D., Hanson, M. A., Cooper, C. & Thornburg, K. L. 2008 Effect of in utero and early-life conditions on adult health and disease. *N. Engl. J. Med.* **359**, 61–73. (doi:10.1056/NEJMra0708473)
- Greene, W. H. 2000 *Econometric analysis*, 4th edn. New Jersey, NJ: Prentice Hall.
- Hutchinson, W. L., Koenig, W., Frohlich, M., Sund, M., Lowe, G. D. O. & Pepys, M. B. 2000 Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. *Clin. Chem.* **46**, 934–938.
- Illi, S., von Mutius, E., Lau, S., Bergmann, R., Niggemann, B., Sommerfeld, C. & Wahn, U. 2001 Early childhood infectious diseases and the development of asthma up to school age: A birth cohort study. *Br. Med. J.* **322**, 390–395. (doi:10.1136/bmj.322.7283.390)
- Jenny, N. S., Yanez, N. D., Psaty, B. M., Kuller, L. H., Hirsch, C. H. & Tracy, R. P. 2007 Inflammation biomarkers and near-term death in older men. *Am. J. Epidemiol.* **165**, 684–695. (doi:10.1093/aje/kwk057)
- Kemp, A. & Björkstén, B. 2003 Immune deviation and the hygiene hypothesis: a review of the epidemiological evidence. *Pediatr. Allergy Immunol.* **14**, 74–80. (doi:10.1034/j.1399-3038.2003.00017.x)
- Kermack, W. O., McKendrick, A. G. & McKinlay, P. L. 1934 Death rates in Great Britain and Sweden: some general regularities and their significance. *Lancet* **226**, 698–703. (doi:10.1016/S0140-6736(00)92530-3)
- King, D. E., Egan, B. M. & Geesey, M. E. 2003 Relation of dietary fat and fiber to elevation of C-reactive protein. *Am. J. Cardiol.* **92**, 1335–1339. (doi:10.1016/j.amjcard.2003.08.020)
- Kuo, H.-K., Bean, J. F., Yen, C.-J. & Leveille, S. G. 2006 Linking C-reactive protein to late-life disability in the National Health and Nutrition Examination Survey (NHANES) 1999–2002. *J. Gerontol. A Biol. Sci. Med. Sci.* **61**, 380–387.
- Libby, P., Ridker, P. M. & Maseri, A. 2002 Inflammation and atherosclerosis. *Circulation* **105**, 1135–1143. (doi:10.1161/hc0902.104353)
- Lohman, T. G., Roche, A. F. & Martorell, R. 1988 *Anthropometric standardization reference manual*. Champaign, IL: Human Kinetics Books.
- Lyon, C. J., Law, R. E. & Hsueh, W. A. 2003 Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology* **144**, 2195–2200. (doi:10.1210/en.2003-0285)
- McDade, T. W. 2003 Life history theory and the immune system: steps toward a human ecological immunology. *Yearb. Phys. Anthropol.* **4**, 100–125. (doi:10.1002/ajpa.10398)
- McDade, T. W. 2005 Life history, maintenance, and the early origins of immune function. *Am. J. Hum. Biol.* **17**, 81–94. (doi:10.1002/ajhb.20095)
- McDade, T., Beck, M. A., Kuzawa, C. & Adair, L. 2001 Prenatal undernutrition, postnatal environments, and antibody response to vaccination in adolescence. *Am. J. Clin. Nutr.* **74**, 543–548.
- McDade, T., Rutherford, J. N., Adair, L. & Kuzawa, C. 2008 Adiposity and pathogen exposure predict C-reactive protein in Filipino women. *J. Nutr.* **138**, 2442–2447. (doi:10.3945/jn.108.092700)
- McDade, T., Rutherford, J. N., Adair, L. & Kuzawa, C. 2009 Population differences in C-reactive protein concentration and associations with adiposity: comparing young adults in the Philippines and the U.S. *Am. J. Clin. Nutr.* **89**, 1237–1245. (doi:10.3945/ajcn.2008.27080)

- Moe, C. L., Sobsey, M. D., Samsa, G. P. & Mesolo, V. 1991 Bacterial indicators of risk of diarrhoeal disease from drinking water in the Philippines. *Bull. World Health Organ.* **9**, 305–317.
- Mueller, C., Buettner, H. J., Hodgson, J. M., Marsch, S., Perruchoud, A. P., Roskamm, H. & Neumann, F.-J. 2002 Inflammation and long-term mortality after non-ST elevation acute coronary syndrome treated with a very early invasive strategy in 1042 consecutive patients. *Circulation* **105**, 1412–1415. (doi:10.1161/01.CIR.0000012625.02748.62)
- Nurgalieva, Z. Z., Malaty, H. M., Graham, D. Y., Almuchambetova, R., Machmudova, A., Kapsultanova, D., Osato, M. S., Hollinger, F. B. & Zhangabylov, A. 2002 *Helicobacter pylori* infection in Kazakhstan: effect of water source and household hygiene. *Am. J. Trop. Med. Hyg.* **67**, 201–206.
- Pearson, T. A. *et al.* 2003 Markers of inflammation and cardiovascular disease: application to clinical and public health practice. *Circulation* **107**, 499–511. (doi:10.1161/01.CIR.0000052939.59093.45)
- Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E. & Ridker, P. M. 2001 C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *J. Am. Med. Assoc.* **286**, 327–334. (doi:10.1001/jama.286.3.327)
- Prado, M. S., Strina, A., Barreto, M. L., Oliveira-Assis, A. M., Paz, L. M. & Cairncross, S. 2003 Risk factors for infection with *Giardia duodenalis* in pre-school children in the city of Salvador, Brazil. *Epidemiol. Infect.* **131**, 899–906. (doi:10.1017/S0950268803001018)
- Radon, K., Windstetter, D., Poluda, A. L., Mueller, B., Mutius, E. v. & Koletzko, S. 2007 Contact with farm animals in early life and juvenile inflammatory bowel disease: a case-control study. *Pediatrics* **120**, 354–361. (doi:10.1542/peds.2006-3624)
- Raqib, R., Alam, D. S., Sarker, P., Ahmad, S. M., Ara, G., Yunus, M., Moore, S. E. & Fuchs, G. 2007 Low birth weight is associated with altered immune function in rural Bangladeshi children: a birth cohort study. *Am. J. Clin. Nutr.* **85**, 845–852.
- Rexrode, K. M., Pradhan, A., Manson, J. E., Buring, J. E. & Ridker, P. M. 2003 Relationship of total and abdominal adiposity with CRP and IL-6 in women. *Ann. Epidemiol.* **13**, 674–682. (doi:10.1016/S1047-2797(03)00053-X)
- Rice, A. L., Sacco, L., Hyder, A. & Black, R. E. 2000 Malnutrition as an underlying cause of childhood deaths associated with infectious diseases in developing countries. *Bull. World Health Organ.* **78**, 1207–1221.
- Rich-Edwards, J. W., Colditz, G. A., Stampfer, M. J., Willett, W. C., Gillman, M. W., Hennekens, C. H., Speizer, F. E. & Manson, J. E. 1999 Birthweight and the risk for type 2 diabetes mellitus in adult women. *Ann. Intern. Med.* **130**, 278–284.
- Ridker, P. M., Buring, J. E., Shih, J., Matias, M. & Hennekens, C. H. 1998 Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* **98**, 731–733.
- Ridker, P. M., Buring, J. E., Cook, N. R. & Rifai, N. 2003 C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14719 initially healthy American women. *Circulation* **107**, 391–397. (doi:10.1161/01.CIR.0000055014.62083.05)
- Rook, G. A. W. 2009 Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. *Immunology* **126**, 3–11. (doi:10.1111/j.1365-2567.2008.03007.x)
- Rook, G. A. W. & Stanford, J. L. 1998 Give us this day our daily germs. *Immunol. Today* **19**, 113–116. (doi:10.1016/S0167-5699(97)01204-8)
- Rook, G. A. W., Adams, V., Hunt, J., Palmer, R., Martinelli, R. & Rosa Brunet, L. 2004 Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. *Springer Semin. Immun.* **25**, 237–255. (doi:10.1007/s00281-003-0148-9)
- Ross, R. 1999 Atherosclerosis—An inflammatory disease. *N. Engl. J. Med.* **340**, 115–126. (doi:10.1056/NEJM199901143400207)
- Sattar, N., McConnachie, A., O'Reilly, D., Upton, M. N., Greer, I. A., Smith, G. D. & Watt, G. 2004 Inverse association between birth weight and C-reactive protein concentrations in the MIDSPAN Family Study. *Arterio. Thromb. Vasc. Biol.* **24**, 583–587. (doi:10.1161/01.ATV.0000118277.41584.63)
- Schulenburg, H., Kurtz, J., Moret, Y. & Siva-Jothy, M. T. 2009 Introduction: ecological immunology. *Phil. Trans. R. Soc. B* **364**, 3–14. (doi:10.1098/rstb.2008.0249)
- Sheldon, B. C. & Verhulst, S. 1996 Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* **11**, 317–321. (doi:10.1016/0169-5347(96)10039-2)
- Strachan, D. P. 1989 Hay fever, hygiene, and household size. *Br. Med. J.* **299**, 1259–1260. (doi:10.1136/bmj.299.6710.1259)
- Tanchoco, C. C., Cruz, A. J., Duante, C. A. & Litonjua, A. D. 2003 Prevalence of metabolic syndrome among Filipino adults aged 20 years and over. *Asia Pac. J. Clin. Nutr.* **12**, 271–276.
- VanDerslice, J., Popkin, B. & Briscoe, J. 1994 Drinking-water quality, sanitation, and breast-feeding: their interactive effects on infant health. *Bull. World Health Organ.* **72**, 589–601.
- Waterland, R. A. & Michels, K. B. 2007 Epigenetic epidemiology of the developmental origins hypothesis. *Annu. Rev. Nutr.* **27**, 363–388. (doi:10.1146/annurev.nutr.27.061406.093705)
- Yazdanbakhsh, M., Kremsner, P. G. & van Ree, R. 2002 Allergy, parasites, and the hygiene hypothesis. *Science* **296**, 490–494. (doi:10.1126/science.296.5567.490)